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Experiments upon the Osmotic Properties of the Living Frog's Muscle

(Three Figures in Text)

A DISSERTATION SUBMITTED TO THE FACULTIES OF THE GRADUATE
SCHOOLS OF ARTS, LITERATURE, AND SCIENCE, IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

BY

ELIZABETH COOKE, PH.D.

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EXPERIMENTS UPON THE OSMOTIC PROPERTIES OF
THE LIVING FROG'S MUSCLE. BY ELIZABETH
COOKE, Ph.D. (Three Figures in Text.)

IN 1894¹ I began a series of investigations with a view to determining what is the rôle that osmosis plays in the animal muscle, and what differences in osmotic phenomena are determined by the different conditions to which living muscles are subjected. These investigations which were carried on during the years 1894 and 1895 form the subject of the following paper.

They have been confined entirely to the muscles of the frog.

Ranke² had shown in 1865 that if one side of a frog's body be tetanised while the other side remains at rest, the muscles of the tetanised side contain more water than the muscles of the resting side, and that the greater the work done the greater was the increase in the contained water. That this was a phenomenon of osmosis, and not a result of increased water formation due to oxidation processes within the muscle, was shown by the fact that in proportion as the muscles gained the blood lost water.

I.

My first set of experiments were made with a view to determining what are the osmotic properties of the resting muscle. I used throughout the gastrocnemius of the frog. By means of sodium chloride solutions of different concentrations I sought for a solution isotonic with the resting frog's muscle, and though there were slight individual differences, owing to the temperature and probably to the season and the condition of the animal, the isotonic solution was found to consist for room temperature of about 0.8 parts by weight

¹ The results which I obtained concerning the effect of fatigue upon the osmotic properties of the muscle have already been mentioned by Prof. Loeb in support of a new theory of the origin of the growth of the muscle through activity. See *Archiv f. d. ges. Physiologie*, LVI. S. 270, 1894.

² *Tetanus*. Leipsic, 1865.

of sodium chloride to 100 volumes of water. The differences observed for muscles from the same individual were nearly always so slight as to fall within the limits of experimental error, while the differences for muscles from different individuals varied for the most part less than 0.1%, the isotonic solution lying usually between 0.75% and 0.85%, though it occasionally rose as high as 0.9%. The method of determination was as follows. The gastrocnemius was removed from the leg, care being taken not to injure the fibres. The tendon of Achilles was cut off near its upper end and in all cases as nearly as possible at the same point. The muscle was placed between two watch-glasses and weighed carefully. It was then placed in a solution of sodium chloride, carefully covered and allowed to remain for one and one-half hours. It was then removed, hastily dried between two bits of filter-paper and re-weighed. The point at which it neither lost nor increased in weight was, as has been stated, for room temperature about 0.8%.

A series of experiments was next made to determine the effect upon the muscle of solutions of sodium chloride having greater and less concentrations than 0.8%. The solutions differed as a rule by 0.1 of one per cent. ranging from 0.05% to 4%. I first investigated whether or not muscles placed in hypotonic solutions took up water according to the laws of osmotic pressure as determined for two solutions having the same concentration as the muscle and the solution used. I was obliged, in this case, to regard the muscle as having the same osmotic pressure as a 0.8% NaCl solution and to estimate the pressure for a solution equal to the difference in concentration between the muscle and the solution in which it was placed. To take a simple case: if a muscle having an osmotic pressure equal to a 0.9% NaCl solution be placed in a solution of 0.5% NaCl, the excess of the osmotic pressure in the muscle over the pressure in the solution is equal to 0.4%, and if the same muscle be placed in a 0.1% solution of NaCl the difference in the two pressures is equal to 0.8%. Hence according to Boyle's law the osmotic pressure exerted by the muscle in the latter case must be twice the pressure exerted in the former case. But besides the difference in pressure the velocity of diffusion will be in the latter case greater than in the former case, and as the two systems will not have reached a state of equilibrium during one and one-half hours this has also to be considered. It was impossible in the cases which I observed to calculate the difference in velocity, since I could not by the methods of which I made use

determine what was the osmotic pressure of the muscle at the end of an hour and a-half, but it could not in any case have been twice as great for the 0.1% solution as for the 0.5% solution. Hence, calling the osmotic pressure of the muscle in a 0.5% solution 1, that of the muscle in a 0.1% solution 2, and the velocity of diffusion in the 0.5% solution 1, the velocity in the 0.1% solution will be something less than 2, hence the amount of water taken up by the muscle in the 0.1% solution must be less than four times the amount taken up in the 0.5% solution. But the amount must be diminished by two factors.

(1) The greater the dilution of a sodium chloride solution the greater the dissociation of the molecules, hence the osmotic pressure of very dilute solutions is always relatively greater than the pressure of less dilute solutions, and instead of the proportion

$$\frac{9-5}{9-1} = \frac{4}{8} \text{ we have } \frac{(9-5)i}{(9-1)i'} = \frac{x}{y} \text{ where } y > 2x.$$

(2) Owing to the greater velocity of diffusion in a 0.1% solution than in a 0.5% solution the internal friction will be greater in the muscle immersed in the 0.1% solution, in consequence of which the amount of water taken up will be correspondingly diminished. Hence an ideal muscle behaving like a sodium chloride solution of 0.9% would, if placed in a 0.1% solution of NaCl, take up an amount of water considerably less than four times the amount which it would take up if placed in a solution of 0.5% NaCl.

The results of investigation demonstrated that the actual muscle took up in a solution of 0.1% more than seven times as much water as in a solution of 0.5%. The results obtained with hypotonic solutions can best be stated in the form of a curve in which the ordinates represent amounts of water taken up, and the concentrations of the sodium chloride be represented by the abscissa line. Fig. 1 shows such a curve constructed from the mean of a number of experiments.

A glance at the curve will show that for solutions near the isotonic point the amount of water taken up increases slowly with the increasing dilution of the solution, but as the solutions become more dilute the amount of water taken up increases at a rate far greater than the rate of dilution of the solution.

This departure of the muscle from the conduct of a sodium chloride solution with which it is isotonic must be due to changes taking place in the muscle in consequence of the taking up of water. As to the nature of these changes it can only be said that the entrance of water

into the muscle cells appears to give rise to dissociation processes in consequence of which the osmotic pressure within the muscle is increased. These dissociation processes do not proceed *pari passu* with

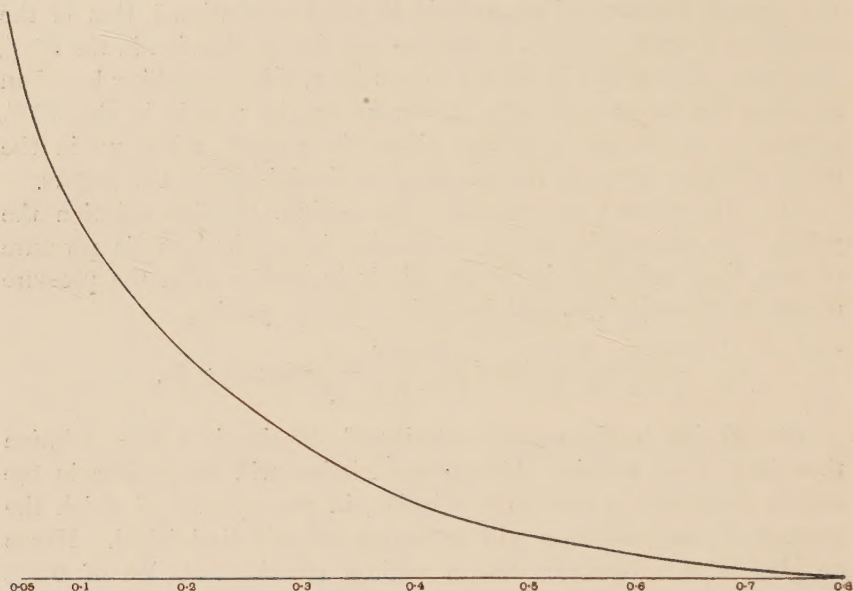


Fig. 1.

an increase in the osmotic pressure of the system, in other words, each increment of water taken up gives rise to an acceleration of the dissociation processes, so that if we represent the osmotic pressure in the muscle in the form of an equation $p = a + f(\chi)$ where a represents the initial pressure, and $f(\chi)$ the pressure due to the increase in the number of ions dependent upon dissociation processes within the muscle, $f(\chi)$ must be considered not as a constant function but as an increasing function.

The loss of water observed in muscles placed in hypertonic solutions by no means followed the same regular course as did the gain of water by muscles placed in hypotonic solutions. Indeed it sometimes happened that a muscle took up water from a solution considerably above the isotonic point. This irregularity, which at first occasioned some surprise, became very clear in the course of experiments presently to be described, which were made to determine osmotic pressure in fatigued muscle. The irregularities grew out of the fact that muscles placed in hypertonic solutions were usually stimulated by the solution

to more or less violent and prolonged contractions, and the osmotic pressure within the muscle, as will be shown later, is powerfully influenced by the production of fatigue substances.

II.

Donders¹ and Hamburger found that solutions isotonic with blood corpuscles at 0° were isotonic with the same at 34°. In the case of solutions of substances which do not dissociate, as for example cane-sugar, this would mean that the osmotic pressure in both systems was increased at the higher temperature by the factor $1 + \alpha t$; while in the case of solutions in which dissociation does take place, if there were any increase in the number of ions in the solution used consequent upon the rise in temperature, there must have been a corresponding amount of dissociation within the blood corpuscle, since the systems in equilibrium at 0° remained in equilibrium at 34°.

Experiments were made to determine whether it was the case for the muscle as Donders and Hamburger had found it to be for the blood corpuscle, that for solutions with which it was isotonic it remained isotonic irrespective of changes of temperature. A number of experiments were made at temperatures varying between 6° and 26° degrees, but the fullest series was made for 9° and 19°. The method was as follows. The two gastrocnemii were removed under conditions as nearly identical as possible, weighed, and the one kept for one and one-half hours in a sodium chloride solution maintained at 9°, while the other was kept during the same time in a sodium chloride solution of the same concentration maintained at 19°. Unlike the results obtained for blood corpuscles it was found that in the muscle the osmotic pressure increases with an increase in temperature by a factor greater than $1 + \alpha t$. In other words, a muscle at 19° took up water from a solution isotonic with a muscle at 9°, thus making evident the fact that with a rise in temperature there takes place an increase in the osmotic pressure within a muscle greater than the increase in osmotic pressure brought about by the same rise in temperature within a solution of sodium chloride. On the other hand, a muscle at 9° lost water to a solution isotonic with a muscle at 19°, that is to say, with a fall in temperature the osmotic pressure within the muscle is diminished by an amount greater than the factor $1 + \alpha t$. This result was indeed to be expected. All that we know of the metabolism of the muscle leads us to believe

¹ *Onderzoekingen gedaan in het physiologisch Laboratorium der Utrechtsch Hoogschool* (3) ix. 26.

that, with a rise in temperature, there takes place an acceleration of certain splitting-up processes within the muscle which would result in an increase in its osmotic pressure.

III.

In order to determine the changes in irritability of the muscle consequent upon the loss or gain of water I made use of the following method. Both gastrocnemii of a frog were removed, and left covered with the skin of the thigh in order to prevent evaporation. The threshold of stimulation being determined for each, one was removed from the skin and placed in a sodium chloride solution, while the other was placed between folds of moist filter-paper and covered with a bell-jar. At the end of an hour and a-half the threshold was again determined. It was found that for the muscle allowed to simply rest for one and one-half hours there was a slight constant decrease in irritability. The muscle immersed in a sodium chloride showed also a decrease in irritability and in the following manner. The irritability was at a maximum with solutions of 0.7% NaCl—though this maximum was less than the irritability of the fresh muscle. It decreased rather slowly with solution 0.7% to 0.4%, when it began to decrease rapidly (0.4% to 0.1%). With solutions above 0.7% the decrease in irritability was a much more rapid one. For muscles which were placed in 0.7% NaCl, and had consequently taken up small quantities of water, the decrease in irritability was less than for muscles which had simply been removed from the frog's body for the same length of time. From this we must conclude that increase of water up to a certain

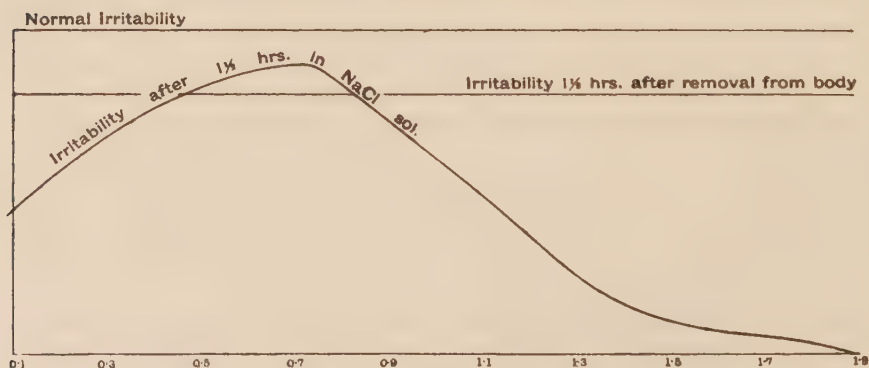


Fig. 2.

point increases the irritability of the muscle; that beyond this point any increase in the amount of water in the muscle decreases the irritability, while loss of water by the muscle always decreases its irritability. These changes are represented in the form of a curve in Fig. 3, where the ordinates represent the irritability of the muscle and the strengths of the solutions are represented upon the abscissa line.

The ordinates for the normal irritability and for the irritability of the muscle which has simply been removed from the frog's body for one and one-half hours being constants these irritabilities will be represented by straight lines parallel to the abscissa. For the muscle immersed in sodium chloride the greatest value of the ordinate is at 0.7%, and this it must be noticed is greater than the value of the ordinate for the muscle which has not been placed in solution.

IV.

The effect upon the resting muscle of salt solutions of different concentrations having been determined I undertook to determine whether, and if so in what way, the osmotic pressure in the muscle is influenced by different forms of activity. In the first series of experiments I investigated the effect of fatigue upon the taking up of water. The method of procedure was as follows: the two gastrocnemii were excised and while one, covered with the skin, was left between moist filter-paper, its fellow was hung up without load, and stimulated for twenty minutes with an interrupted induction current. During the first part of the stimulation the muscle remained strongly contracted, and then gradually relaxed to its original length. The two muscles were then weighed and each was placed in a dish containing a solution of sodium chloride. It was found in every case that the osmotic pressure had been increased in the tetanised muscle. The following table contains the figures obtained when the muscles had been immersed in a solution of 0.2% NaCl at a temperature of 22°.

		Weight in mgs.	Gain in wt.	% of gain			Weight in mgs.	Gain in wt.	% of gain
I.	{ Resting M.	2932	356	11.8	IV.	{ R. M.	1007	313	31
	{ Fatigued M.	2850	520	18.2		{ F. M.	1031	489	47
II.	{ R. M.	1235	315	25	V.	{ R. M.	4069	544	13
	{ F. M.	1225	474	38		{ F. M.	4021	689	17
III.	{ R. M.	1150	194	17	VI.	{ R. M.	4805	531	11
	{ F. M.	1147	332	29		{ F. M.	5049	824	16

In a series of control experiments where the two gastrocnemii were submitted immediately after removal to the same solution of NaCl, the maximum differences in the amount of water taken up by any two muscles from the same frog amounted to 1% of the original weight. Here the differences amount to as much as 18% and they are always in the same sense, namely, an excess in the amount taken up by the fatigued muscle. In the case of hypertonic solutions this process, as was expected, was reversed, *i.e.* the fatigued muscle lost less water than its fellow. The isotonic solution was of course no longer the same for both muscles, and in a 1% solution the fatigued muscle still continued to take up water, the isotonic solution being apparently about 1.1%, which if 0.8% be taken as the normal, representing a difference of 0.30% in the solutions of NaCl isotonic for resting and for fatigued muscles.

It will be seen from the preceding table that individual pairs of muscles show great differences in the amount of water taken up. As has been previously stated these differences appear to be dependent, in a measure, upon the season and the condition of the frog, but the greatest differences are due to differences in the size of the muscle. In the case of large muscles the internal mass of the muscle is greater in proportion to its external surface than in the case of small muscles, consequently the rate of diffusion as compared to the mass of the muscle would be much less in the case of the large than in the case of the small muscle. Hence it follows that the amount of water lost or taken up by the muscle is a function not simply of the mass of the muscle or of its surface but of the proportion between these two.

V.

I made a number of experiments to determine the effect of the load and of the time of stimulation upon the osmotic pressure within the muscle. As to the effect of the load I found that the increase in osmotic pressure varies with the load in the same way that the ability to do work varies with the load, *i.e.* an increase in the load up to a certain point determines an increase in the osmotic pressure, while beyond this point an increase in the load determines a decrease in osmotic pressure. Whether or not the point at which the maximum increase in osmotic pressure is obtained is identical with the optimum load for the working muscle I did not attempt to determine. As to the effect of the time of stimulation upon the osmotic pressure I

found that up to the point of fatigue the osmotic pressure increases, but this increase by no means varies directly with the time, being most rapid at the beginning and increasing very little towards the point of complete fatigue. The character of the increase is represented graphically in the following curve, Fig. 3, the time of stimulation being marked upon the abscissa line and the increase in the osmotic pressure being represented by the ordinates.

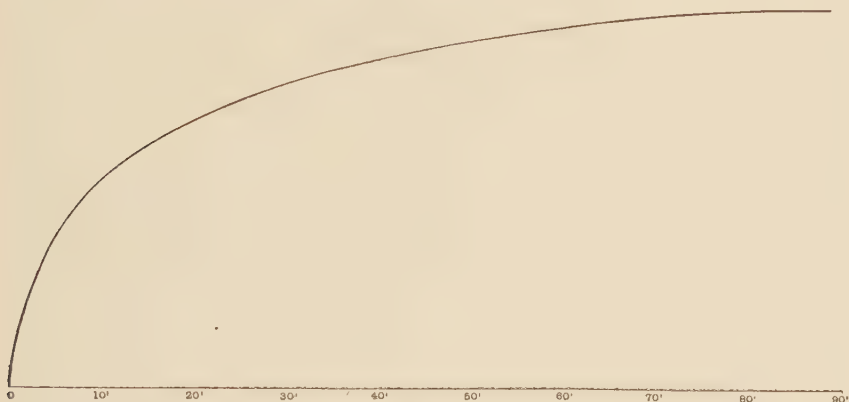


Fig. 3. Illustrating the rate at which the taking up of water varies with the time of stimulation of the muscle.

Identical experiments were made upon muscles stimulated by single shocks instead of by the interrupted current. It was found that a muscle which had performed work by means of single contractions had, like a tetanised muscle, a higher osmotic pressure than a resting muscle; that the pressure increased with the load up to a certain point and beyond this point decreased with any addition of the load. The amount of load necessary to bring about a maximum increase in the osmotic pressure within the muscle was found, however, to be much greater when the muscle was stimulated with single shocks than when it was tetanised. As was the case in the tetanised muscle the increase in osmotic pressure continued up to the point of complete fatigue. However, the curve representing the increase in osmotic pressure with the time of work is not the same if the work be performed by means of single contractions as if it be performed by tetanic contractions. In the former case the increase in osmotic pressure is not so rapid during the first few moments of stimulation, but increases much more gradually during the first half-hour and then very slowly up to the point of fatigue.

VI.

The question naturally arose as to whether the foregoing changes in the osmotic pressure of the muscle were a result solely of changes in the muscle due to muscular work, or whether they might be due wholly or in part to the passage of an electric current through the muscle. In order to determine whether or not the electric current in itself played any rôle I hung up a pair of muscles, determined the threshold of irritability, and allowed a current slightly weaker than one which gave the faintest possible contraction to flow through one muscle during one and a-half hours while the other muscle remained at rest.

There never was the slightest change in the osmotic pressure due to the passage of the electric current, but the possibility still remained that an electric current too weak to cause a contraction might be without effect upon the muscle while a stronger current would not be indifferent. To investigate this possibility I hung up two muscles with the same load, and stimulated both to fatigue by means of the same electric current. I then removed one muscle from the circuit and allowed the current to pass through the other for an hour and a-half, but without obtaining any differences in the osmotic pressure of the two sets of muscles. Hence we must conclude that the electric current, as such, is unable to determine any of those decompositions in the muscle which lead to variations in its osmotic pressure.

VII.

In the preceding experiments I investigated some of the changes which take place in the muscle when it performs mechanical work. These changes, as has already been stated, consist in an increase in the number of ions within the muscle in consequence of which its osmotic pressure is increased. It seemed an *à priori* conclusion that a like increase in osmotic pressure would be determined by internal work of the muscle, and such indeed I found to be the case.

I arranged two muscles for stimulation, one with a light load and the other with a load of four kilos, which was far heavier than the maximum load for the muscle. The four kilos were supported upon a horizontal bar in such a way that the smallest shortening of the muscle must lift them from their support. The muscles were then stimulated until the one which was able to contract was fatigued and both were then placed in a solution of sodium chloride. The muscles which had

done no mechanical work had the same osmotic pressure as those which had done mechanical work. That the former were completely fatigued was demonstrated by the fact that after removal of the load no contraction could be brought about by further stimulation. Thus it may be stated, that in so far as the decompositions resulting from complete fatigue can be determined from the changes in the osmotic pressure of a muscle, those decompositions are quantitatively equal whatever be the source of the fatigue.

In these experiments I dealt with the internal work of the muscle when stimulated to contract against a load which it was unable to lift, but there remained another kind of internal work to be investigated, namely, the work done by a muscle in its resistance to being stretched out by a load. I hung up two muscles, one of which remained without load and to the other of which I suspended a load during one hour. I varied the load between 100 and 1400 grams; I found that for the muscles that I used, and which weighed as a rule about one gram, no change in the amount of water taken up from an 0.4 % NaCl solution was determined by loading up to the point where the load neared 200 grams. At that point a slight increase in the amount of water taken up by the loaded over the non-loaded muscle began, and this increase continued slowly up to about 500 grams, and from that point more rapidly up to 1400 grams.

This increase in the amount of water taken up might result from either of two causes. First, it might be owing to chemical changes in the muscle which resist the stretching out, *i.e.*, to an increase in osmotic pressure consequent upon internal work, or it might be due to a purely physical cause, namely, an increase in the size of the interstitial spaces of the muscle consequent upon the stretching to which it had been subjected. If the latter were the cause it must occur that stretching out a muscle after it had been fatigued, *i.e.*, after no further increase in the osmotic pressure of the muscle was possible, must still result in an increase in the amount of water taken up by the muscle.

To ascertain whether or not this were the case I fatigued two muscles in the following manner: I stimulated both under a load of 150 grams for twenty minutes, then for twenty minutes under a load of 70 grams, and for twenty minutes longer without load. At this point though both muscles were completely fatigued they had returned to their original length. One muscle was then loaded with weights varying from 700 to 1000 grams and the other muscle remained

suspended without load. At the end of an hour they were placed in 0.4 % NaCl solution and after an hour and a-half the amount of water taken up was in all cases nearly the same, varying slightly first on one side then on the other.

SUMMARY AND CONCLUSION.

The chief points demonstrated in the course of these experiments may be briefly recapitulated as follows.

1. A muscle immersed in a hypertonic sodium chloride solution does not behave according to the laws of osmotic pressure governing two solutions having the pressure of the immersing fluid and the fluid which is isotonic for the muscle, but owing doubtless to splitting-up processes within itself consequent upon the taking up of water the muscle behaves like a solution having a higher osmotic pressure than the solution for which it is isotonic.

2. A rise in temperature determines an increase in the osmotic pressure within the muscle, and this increase is greater than the increase which would result from the given rise in temperature in a solution the molecules of which do not dissociate.

3. In a muscle which has been removed for some time from the body a loss of water decreases the irritability of the muscle, and a taking up of water, up to a certain point, increases the irritability; beyond that point the taking up of water decreases the irritability of the muscle.

4. A muscle which has done work has a higher osmotic pressure than a resting muscle. The greater the fatigue the greater the increase in osmotic pressure.

5. The increase in the osmotic pressure consequent on fatigue is due not to any change in the physical properties of the muscle but to chemical changes within the muscle.

For the sake of simplicity I have hitherto refrained from speaking of the fact that in the preceding experiments I had to deal not only with phenomena of osmosis but with phenomena of diffusion as well, since the muscle tissues are to some extent permeable for the molecules of salts. Nevertheless, this fact in no way invalidates the foregoing conclusions, since the observations were made by determining, in the first place, a solution isotonic for the muscle, and then comparing its behaviour in solutions of greater and less concentration with its behaviour in the isotonic solution. However, although it was not requisite for my purpose to determine what constituents were lost by

the muscle, it is conceivable that in regard to one point with which I have dealt, namely, irritability, this question may be of importance, and it is my intention in a set of experiments which I purpose to carry out to decide this point more definitely. I also hope to investigate the result of changes of temperature more fully, particularly to do a number of experiments at very low temperatures.

The facts which I have thus far been able to determine seem to me interesting chiefly as being a slight increase in our knowledge of the behaviour of the muscle in relation to one set of forces, namely, osmotic forces. That osmotic forces play a very great rôle in actual life phenomena there can be no doubt, but whether or not osmotic phenomena are of great significance in the muscle, and if so in what way they are significant, must be for the most part determined by future investigations. I have already alluded to Loeb's¹ theory of the origin of growth through activity. It may very well be as he supposes that growth is brought about by the increase in osmotic pressure consequent upon the dissociations arising from muscular work. Koeppe² has recently pointed out that contrary to our hitherto received ideas the salts furnish a supply of energy to the body. If Loeb's theory be correct this molecular energy is instrumental not only in transporting material from place to place but in bringing about growth.

That osmosis plays a rôle in phenomena of irritability has been shown by Loeb³ in his experiments upon the ova of sea-urchins. He was able at will to accelerate or retard segmentation by decreasing or increasing the concentration of the sea water. Schively⁴ through the same means was able to quicken or to slow the respiratory movements of fundulus. My experiments show that the muscle cell obeys the same law, namely, that an increase in the water in the cell within certain limits determines an increase in irritability, while a diminution in the water of a cell determines a decrease in its irritability.

In conclusion I wish to express my sincerest thanks to Professor Loeb, whose advice and encouragement have been of invaluable assistance to me throughout this work.

¹ *Archiv f. d. g. Physiologie*, LVI. s. 567, 1894.

² *Archiv f. d. g. Physiologie*, LXII. s. 567, 1896.

³ *Journal of Morphology*, VII. p. 25, 1892.

⁴ *Archiv f. d. g. Physiologie*, LIV. s. 531, 1893.

